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FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 48, No. 4, 2010
pp. 637-645

Glycophenotype of prostatic carcinomas

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Abstract: The factors that affect the progression of prostatic carcinoma are poorly understood, but it is known that carbohydrate antigens on the tumour cell surface play a role in the transforming and metastatic processes. The present report aimed to perform a comparative, lectin-histochemical study of benign and carcinomatous prostates, using a battery of lectins, in combination with monoclonal antibodies against Lewis antigens, and a semi quantitative study, to investigate the changes in glycosylation patterns that occur in prostatic carcinoma. Blocks from 27 necropsy cases of prostatic carcinoma were sectioned and stained with H+E, fifteen biotinylated lectins chosen to probe for a wide range of oligosaccharide sequences within several categories of glycoprotein glycans, using a lectin-biotin avidin-peroxidase method, and monoclonal antibodies against Lewis^a, sialyl Lewis^a and sialyl Lewis^x antigens. The glycophenotype of prostatic carcinoma differed from that of the noncancerous prostate in revealing more intense staining with the following lectins (AAA, UEA-1, DBA, WFA, VVA, HPA, BSA-1$_{B4}$, MPA, ECA, AHA, and CTA), while the binding patterns of (GNA and NPA) were almost similar in both prostatic carcinoma and the noncancerous prostate. Lewis antigens are found to be expressed in prostatic carcinomas but not in the noncancerous prostate. The observations of this study suggest that the gylcophenotype of transformed prostatic cells was modified. It showed a moderate increase in, and changing patterns of, fucosylation and galactosylation, increased branching of side chains and sharp rise in 2 deoxy, 2 acetamido galactosylation and masking process by sialylation, especially by α2-3 and α2-6 linkages. All these changes in the glycosylation pattern of the transformed prostatic cells were observed on O-glycans, no changes were observed on N-glycans.

Key words: prostatic carcinoma, Lewis antigens, lectin

Introduction

The factors that affect the progression of prostatic carcinoma are poorly understood, but it is known that carbohydrate antigens on the tumour cell surface play a role in the transforming and metastatic processes [1]. Although numerous "biochemical tumour markers" have been investigated as potential markers for detecting the capacity for progression of prostatic carcinoma, including lectins and monoclonal antibodies against many carbohydrate antigens, to date there are no reports of tumour-associated antigens in prostatic carcinoma. Without exception, these markers proved disappointing in that they do not give consistent results in all cases, and are not specific for, or unique to, prostatic carcinoma. Most of these studies failed to map the sequential steps in the synthesis of the type I and II chain carbohydrate structures, which bind to different markers of transformed prostatic cells. The changes in glycosylation that occur in transformed cells can take a variety of forms. Examples have been found of loss of expression or excessive expression of certain structures, the persistence of incomplete or truncated structures, the accumulation of precursors, and, less commonly, the appearance of novel structures [2,3]. Most of the studies that analysed the glycans phenotype of prostatic carcinoma also failed to relate the staining pattern to the chemical structures.

Because the significant morbidity and mortality associated with prostate cancer can universally be attributed to the consequences of metastases; the goal of the present report was to perform a comparative, lectin-histochemical study of noncancerous prostate and carcinoma of prostate with at least two metastasises.

A battery of 19 markers (most of them had not been used in previous studies) including 15 lectins in

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Folia Histochem Cytobiol. 2010:48(4): 637 (637-645)
Doi: 10.2478/v10042-010-0089-9

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combination with three monoclonal antibodies against Lewis antigens and polyclonal antibody against prostate specific antigen were used in a semi quantitative study to investigate the changes in glycosylation patterns occur in prostatic carcinoma.

Materials and methods

Ten formalin-fixed, paraffin-embedded specimens of grossly non-cancerous human prostate (normal prostate, prostate hypertrophy and prostatitis) and twenty seven cases of high grade prostatic carcinoma (8-10 Gleason score) surgically excised in a 20-year period prior to radio therapeutic, chemotherapeutic and hormonal manipulation regimes, are obtained from the histopathology archive of the Manchester Royal Infirmary.

The mean age of the cases was 72 years (range 50 to 92 years). Twenty seven of prostatic carcinoma had metastases; deposits were present in the following sites: lymph nodes (18), bone (14), lung (16), liver (12), adrenal (8), pituitary (3), pancreas (2), spleen (2), bladder (3), kidney (2), cerebellum (1), thyroid (2), and breast (1).

Lectin histochemistry. Sections were deparaffinised, the endogenous peroxidase was blocked in methanolic hydrogen peroxide and they were then hydrated through graded ethanols to water. They were pre-treated with a solution of 0.1% (w/v) trypsin (type II crude, from porcine pancreas, Sigma Chemical Co.) in 0.05 M TRIS buffered saline (TBS), pH 7.6 containing 0.1% (w/v) calcium chloride. After washing, they were stained with the panel of 15 biotinylated lectins (Table 1) using a lectin-biotin avidin-peroxidase method [4], with DAB as the disclosing agent and a methyl green counter stain. Most biotinylated lectins were obtained from the Sigma Chemical Company, MAA and SNA from Boehringer Mannheim. All were used at a concentration of 10 μg/ml apart from MAA and SNA, which were used at 50 μg/ml.

Negative control sections were included in every staining run with buffer replacing the lectin and, where possible, controls were carried out using the appropriate competing sugars. Positive controls were also carried out, variously by using other tissues within the section or by using known 'positive' slides from blocks of other tissues.

Digestion with dilute acid was performed by incubating additional sections in diluted sulphuric acid "0.1M H_2SO_4" (BDH) for 24 hours at 4°C, prior to staining with 15 lectins. This digestion was used as a control for the staining by SNA and MAA and also to determine the sub-terminal sugars.

Beta-elimination was performed, before staining with 15 lectins, based on the method of Downs *et al* [5].

Immunohistochemistry. A panel of antibodies including polyclonal antibody against prostate specific antigen "PSA" (Dako), and monoclonal antibodies against Lewis^a (MAb Le^a), sialyl Lewis^a (MAb sLe^a) (Novocastra Laboratories Ltd.) and sialyl Lewis^x (MAb sLe^x) (Chemicon International Ltd.) glycans (Table 1) was applied to the same cases of prostatic carcinoma. Sections (5 χm) were de-waxed, the endogenous peroxidase was blocked in methanolic hydrogen peroxide, and they were then hydrated through graded ethanols to water. They were microwaved for 5 minutes in low power (106 w) after boiling the sodium citrate buffer pH 6.0. After washing, non-specific staining was blocked by incubating the slides in diluted normal goat serum (NGS) for 20 minutes, followed by treatment with primary antibody diluted in 0.5-1% (w/v) BSA (bovine serum albumin) in TBS + 0.01% (w/v) sodium azide for 60 minutes at room temperature 17-21°C. After washing in two changes of TBS, slides were treated with secondary antibody, 1/300 (v/v) biotinylated goat anti-mouse diluted in 0.5-1% (w/v) BSA in TBS + 0.01% (w/v) sodium azide, and incubated for 40 minutes at room temperature then rinsed as before in TBS. The slides were treated with 0.2% v/v streptavidin peroxidase diluted in TBS for 30 minutes at room temperature. After washing in TBS, DAB (3,3'diaminobenzidine tetrahydrochloride) was applied for 5-10 minutes and the slides counter-stained in Mayer's haematoxylin.

The tissue components analysed were stroma, prostatic ducts, epithelium (basement membrane, luminal secretions, cytoplasm, apex, apical secretion, apical membrane, lateral membrane, supranucleus, nuclear membrane and nucleoplasm). In some cases of prostatic carcinoma, it was not possible to analyse glandular epithelial cells in such detail because of cellular pleomorphism and irregular acinar formation.

Results

The current study showed PSA is present in the cytoplasm of benign and malignant prostate epithelium of all cases.

Lectin specificity and the validity of the lectin histochemistry were confirmed by the different controls. There was a different staining of noncarcinous prostatic tissue and prostatic carcinomas with lectins and antibodies, the main findings are summarised and arranged in groups according to the markers and their ligand carbohydrate structures in Table 2. The experiments with the inhibitory sugars completely blocked the lectin binding.

Normal prostatic tissue

In the normal prostate AAA, UEA-1 and DBA bound weakly to the membranes of less than 10% of elective cell population of epithelial cells. Monoclonal antibodies against Lewis antigens showed almost no binding except MAb Le^a that bound to less than 10% of elective cell population of epithelial cells. WFA showed no binding, whereas VVA and HPA bound weakly to a few cells. BSA-1_{B4} revealed strong staining pattern in up to 10% of normal epithelium. MPA, ECA and AHA demonstrated strong binding to selective epithelial cell and luminal secretions, and were negative in some cases, while CTA was weakly bound in some cases. GNA and NPA illustrated a moderate to strong binding pattern in the epithelium. SNA and MAA demonstrated a moderate binding to the cytoplasm and membranes of the epithelium.

Malignant prostatic tissue

AAA, UEA-1, DBA, MAb Le^a, MAb sLe^a and MAb sLe^x. The number of prostatic carcinoma cases that bound to the AAA, UEA-1, DBA, MAb Le^a, MAb sLe^a and MAb sLe^x markers varied in regard to site, proportion of positive malignant cells and the pattern of staining. Approximately 70% of the cases of prostatic carcinoma bound to AAA, 51% to UEA-1, 37% to DBA, 78% to MAb Le^a, 78% to MAb sLe^a and 82%

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Doi: 10.2478/v10042-010-0089-9

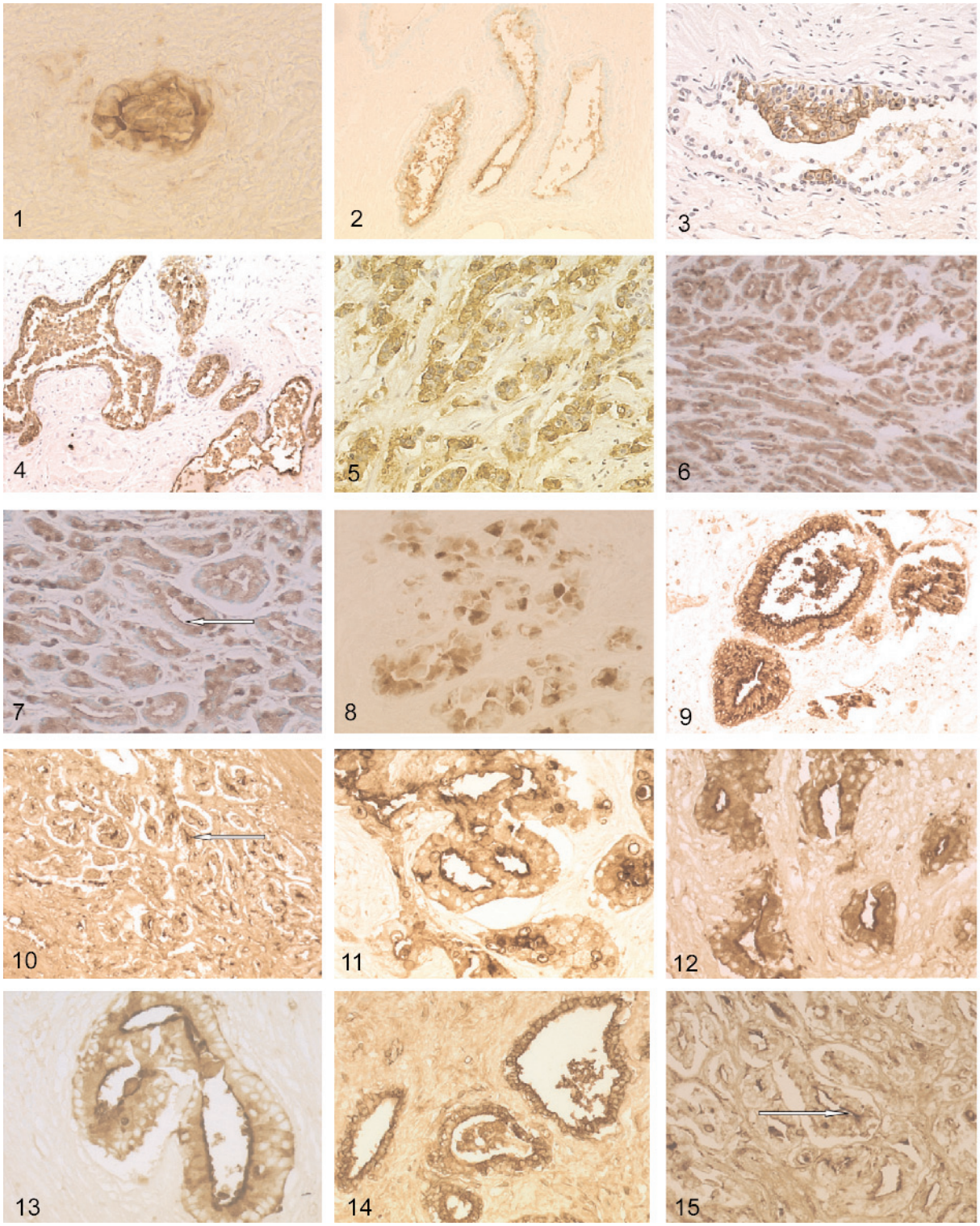

Fig. 1. UEA-1 staining profile of prostatic carcinoma (original magnification ×40). **Fig. 2.** DBA staining profile of prostatic carcinoma (original magnification ×10). **Fig. 3.** MAb Le^a staining profile of prostatic carcinoma (original magnification ×20). **Fig. 4.** MAb sLe^a staining profile of prostatic carcinoma (original magnification ×10). **Fig. 5.** MAb sLe^x staining profile of prostatic carcinoma (original magnification ×20). **Fig. 6.**1 WFA staining profile of prostatic carcinoma (original magnification ×10). **Fig. 7.** HPA staining profile of prostatic carcinoma (original magnification ×20). **Fig. 8.** VVA staining profile of prostatic carcinoma (original magnification ×20). **Fig. 9.** BSA-1B4 staining profile of prostatic carcinoma (original magnification ×10). **Fig. 10.** MPA staining profile of prostatic carcinoma (original magnification ×10). **Fig. 11.** AHA staining profile of prostatic carcinoma (original magnification ×20). **Fig. 12.** ECA staining profile of prostatic carcinoma (original magnification ×20). **Fig. 13.** CTA staining profile of prostatic carcinoma (original magnification ×40). **Fig. 14.**3 SNA staining profile of prostatic carcinoma (original magnification ×20). **Fig. 15.** MAA staining profile of prostatic carcinoma (original magnification ×20).

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Folia Histochem Cytobiol. 2010:48(4): 639 (632-636)
Doi: 10.2478/v10042-010-0070-7

Table 1. Specificities of lectins (agglutinins) and monoclonal antibodies.

Abbreviation	Marker name	Carbohydrate specificity
MAb Le^a	Monoclonal antibody against $Lewis^a$ antigen	Galβ1-3 (Fuc α1-4) GlcNAcβ1-3Gal-
MAb sLe^a	Monoclonal antibody against sialyl $Lewis^a$ antigen	NeuAc(α2-3) Galβ1-3 (Fuc α1-4) GlcNAcβ1-3Gal-
MAb sLe^x	Monoclonal antibody against sialyl $Lewis^x$ antigen	NeuAcα2-3Galβ1-4 (Fucα1-3)GlcNAcβ1-3Gal-
AAA	*Anguilla anguilla agglutinin*	α-L-fucosyl termini and fucosylated type I chains
UEA-I	*Ulex europaeus agglutinin*	L-Fucα1,2Galβ1,4GlcNAcβ1-
DBA	*Dolichos biflorus agglutinin*	GalNAcα1,3(L-Fucα1,2) Galβ1,3/ 4GlcNAcβ1-
WFA	*Wisteria floribunda agglutinin*	GalNAcα1,6Galβ1-> GalNAcα1,3Galβ1-
VVA	*Vicia villosa agglutinin*	GalNAcα1,3 Gal- > GalNAcα1,6 Gal-
HPA	*Helix pomatia agglutinin*	GalNAcα1-
$BSA\text{-}1_{B4}$	*Griffonia simplicifolia agglutinin*	Galα1- & Galα1,3Gal-
MPA	*Maclura pomifera agglutinin*	Galβ1,3GalNAcα1- & GalNAcα1> Galα1
ECA	*Erythrina cristagalli agglutinin*	Galβ1,4GlcNAcβ1-(and Galα1,3Galβ1,4 GlcNAcβ1-)
AHA (PNA)	*Arachis hypogaea*/peanut *agglutinin*	Galβ1,3GalNAcα1-> Galβ1,4GlcNAcβ1-
CTA	*Erythrina corallodendron agglutinin*	Galβ1,4GlcNAc- especially in multiple branches
SNA	*Sambucus nigra agglutinin*	NeuAcα2,6Gal/GalNAc-
MAA	*Maackia amurensis agglutinin*	NeuAcα2,3Galβ1-
GNA	*Galanthus nivalis agglutinin*	Non-reducing terminal α-D-Man, especially Man-α1,3Man linkage
NPA	*Narcissus pseudonarcissus agglutinin*	Non-reducing terminal α-D-Man, especially Man-α1,6Man linkage
LTA	*Tagonolobus purpureus agglutinin*	α-L-fucosyl termini (especially where clustered) and fucosylα1,6 links on N-acetyl chitobiosyl core sequences of N-glycan
SBA	*Glycine max/soybean agglutinin*	GalNAcα1- more than Galα and GalNAcβ1-

Fuc: fucose; Gal: galactose; GlcNAc: 2 deoxy, 2 acetamidoglucosyl residue; GalNAc: 2 deoxy, 2 acetamidogalactosyl residue; Man: mannose; NeuAc: sialic acid

to MAb sLe^x. In most cases, less than 5% of the tumour cells (glandular and ductal epithelium) bound to the above lectins, but in a few cases up to 70% of tumour cells bound to AAA and UEA-1. Almost one third of the prostatic carcinomas expressed Lewis antigens in up to 80% of the tumour cells. The markers above showed a granular pattern of staining, mostly apical and membranous, within certain cell populations of the malignant prostatic epithelium of the tumours (Figs 1, 2, 3, 4 and 5). They also showed patches of staining within the stroma.

WFA, VVA and HPA. WFA bound strongly to luminal secretions and the apices of epithelial cells within the malignant prostatic epithelium (glandular and ductal) and also bound moderately to stroma in a patchy manner. This staining profile was present in all tumours (Fig. 7), irrespective of grade of prostatic carcinoma. Some areas of poorly differentiated carcinoma showed a diffuse cytoplasmic pattern of staining. The intensity of stain was inconsistent in some parts of the tumours in some cases (focal). HPA and VVA gave variable results, showing binding by 24 and 21 prostatic carcinomas respectively. The HPA staining pattern resembled the WFA staining profile in most of the prostatic carcinomas, but with a wide degree of variation in regard to intensity and proportion of positive cells (Fig. 8). In contrast, VVA gave a patchy pattern of staining with a strong tendency to bind to subsets of cells (Fig. 9). It bound to less than 40% of the malignant epithelium in the prostatic carcinomas.

$BSA\text{-}1_{B4}$. $SA\text{-}1_{B4}$ gave unexpected results. It was expected to be negative, but appeared to be positive in 19 of the prostatic carcinomas (Fig. 10). The staining pattern, which was mostly selective and granular, was concentrated in the apex, the membranes and the putative location of the Golgi apparatus.

MPA, ECA, CTA and AHA. MPA bound strongly in a uniform pattern to the vast majority of malignant prostatic epithelia in 26 out of 27 prostatic carcinomas. It showed a strong granular cytoplasmic stain especially in the apex, apical secretions and membranes in prostatic carcinomas (Fig. 11). In comparison, AHA

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Folia Histochem Cytobiol. 2010:48(4): 640 (637-645)
Doi: 10.2478/v10042-010-0089-9

Table 2. Lectin and monoclonal antibodies binding patterns of normal and cancerous prostatic tissues

Markers	Noncancerous prostate			Prostatic carcinomata (total number of cases = 27)				
	Luminal secretion	Secretory cells		Luminal secretion	Secretory cells		Percentage of positive cells	Percentage of positive cases
		cytoplasm	membranes		cytoplasm	membranes		
MAb Le^a	-	-	++ (<10%)	+++	+++	++++	1%	78%
MAb sLe^a	-	-	-	++++	+++	++++	<40%	78%
MAb sLe^x	-	-	-	++++	+++	++++	<60%	82%
AAA	-	+	+ (<10%)	+++	++++	++++	<70%	70%
UEA-I	-	+	+ (<10%)	+++	+++	++++	<50%	51%
DBA	-	-	+ (<10%)	+++	+++	++++	<5%	37%
WFA	-	-	-	++++	+++	++++	70-100%	100%
VVA	++	-	+	++++	+++	++++	<40%	78%
HPA	-	-	+	+++	++++,+++	++++	1-90%	89%
$BSA\text{-}1_{B4}$	-	-	++(<10%)	+++	++++	++++	<60%	70%
MPA	+++	+++	+++	++++	+++	++++	50-100%	96%
ECA	+++	+++	+++	++++	++++	++++	25-100%	100%
AHA (PNA)	+++	+++	+++	++++	+++	++++	20-100%	96%
CTA	+	+	+	++++	+++	++++	10-100%	96%
SNA	-	++	++	++++	+++	++++	50-100%	96%
MAA	-	++	++	++++	+++,++	++++	1-85%	89%
GNA	-	+++, ++	+++, ++	++	+++	++	40-80%	100%
NPA	-	+++, ++	+++, ++	++	+++	++	50-95%	100%

The staining intensity of lectin reaction was graded as, ++++: very strong binding; +++: strong binding; ++: moderate binding; +: weak binding; -: negative staining.

showed a focal staining profile, with several degrees of intensity. The AHA staining profile also showed some variation from MPA in regard to the proportion of positive tumour cells in prostatic carcinomas (Fig. 12). ECA showed the same staining pattern as AHA, and bound to the majority of tumour cells (Fig. 13). In comparison to AHA and ECA, CTA gave an inconsistent staining pattern with variable proportions of positive neoplastic cells (Fig. 14).

SNA and MAA. SNA showed very strong granular staining patterns covering all parts of the neoplastic epithelial cells, except the nucleoplasm, in the vast majority of cases of prostatic carcinomas (Fig. 15). In comparison, the MAA staining profile was variable, in respect of intensity, among the tumour cells. It differed from case to case, yielding a patchy staining pattern in some cases and a focal staining pattern in others (Fig. 16). It appeared that fewer than 60% of the tumour cells bound to MAA in most of the cases of prostatic carcinoma.

GNA and NPA. Both GNA and NPA showed the same staining pattern, demonstrated by moderate to strong, granular, cytoplasmic staining, mostly apical and in the supra-nucleus, which did not change after β-elimination, but slightly increased after dilute acid.

Pre-treatment by β-elimination and dilute acid. Pre-treatment by β-elimination revealed a dramatic loss in both cellular and stromal staining of most lectins, except a moderate increase in MAA-staining, in some tumours, but a decrease in others, while SNA staining remained constant or was increased (Table 3). In contrast, pre-treatment with dilute acid gave a considerable increase in both cellular and stromal staining of all lectins except SNA-staining remained unchanged (Table 3).

Discussion

In this study, the first group of markers AAA, UEA-1, DBA, MAb Le^a, MAb sLe^a and MAb sLe^x demonstrated the presence of several fucosylated glycans of type I and type II chains in prostatic carcinomas. These findings are consistent with the observations of previous lectin studies [6-8], except for McMahon *et al* [9] who found no staining with the lectins directed against fucosylated structures (UEA-1 and LTA), and Loy *et al* [10], McNeal & Alroy *et al* [11] and Drachenberg & Papadimitriou [12] who found no staining with DBA. These exceptions can be explained by understanding the possible biochemical pathway of the synthesis of

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Doi: 10.2478/v10042-010-0089-9

Table 3. Effects of pre-treatment by β elimination and dilute acid pre-treatment (removing sialic acid).

Lectin	Pre-treatment by β elimination		Dilute acid pre-treatment	
	Tumor	Stroma	Tumor	Stroma
AAA	↓↓	↓↓	↑↑↑	↑↑
UEA-1	↓↓↓	↓	↑↑	↑↑
DBA	↓↓	↓↓↓	↑↑	↑↑
WFA	↓↓/↑	↓↓↓	↑/=	↑/=
VVA	↓↓↓	↓↓↓	↑↑	↑
HPA	↓↓	↓↓	↑↑↑	↑
BSA-1_{B4}	↓/↑	↓	↑↑↑	↑↑
MPA	↓↓↓	↓↓↓	=	=
ECA	↓↓	↓↓	↑↑	↑↑
AHA	↓↓	↓↓	↑↑↑	↑↑
CTA	↓↓	↓↓	↑↑↑	↑↑
SNA	↓↓	↓↓	=	=
MAA	↓↓/↑	↓↓↓	↑/=	↑/=
GNA	=	↓↓↓	↑/=	↑/=
NPA	=	↓↓↓	↑/=	↑/=

↓ = Slight decrease in stain; ↓↓ = moderate decrease in stain; ↓↓↓ = sharp decrease in stain; ↑ = slight increase in stain; ↑↑ = moderate increase in stain; ↑↑↑ = sharp increase in stain; =: no change; ↑/= = slight increase in staining of some cases and unchanged in others; ↓/↑ = Slight decrease in staining of some cases and slight increase in staining of others; ↓↓/↑ = moderate decrease in staining of some cases and slight increase in staining of others

UEA-1, AAA and DBA acceptors, which starts with the fucosylation of the disaccharide precursor core (Galβ1-3/4GlcNAc) by α1, 2-fucosyltransferase to become Fucα1-2Galβ1-3/4GlcNAcβ1- (UEA-1 or AAA ligand). The next step is the addition of GalNAc residues by a specific 2 deoxy, 2acetamidogalactosyltransferase to produce GalNAcα1-3(Fucα1-2) Galβ1-3/4GlcNAcβ1- (DBA ligand).

In respect of Lewis antigens, the results of this study showed low expression of Le^a [13-15]. However, a comprehensive search of the available literature has found only one study investigating the expression of sLea antigen. Zhang *et al* found that one prostatic carcinoma out of five expressed sLe^a in more than 50% of the tumour cells [16], but they did not report the level of expression in the other cases, therefore, their findings are not inconsistent with the results of this present study. Nevertheless, Jorgensen *et al* [17], Martensson *et al* [18], Jorgensen *et al* [19] and Idikio [20] found up-regulation of sLe^x expression, which is also consistent with the observations of this present study, and they associated this with increased potential for tumour progression and poor prognosis.

The results of WFA, VVA and HPA, before and after pre-treatment with dilute acid, confirmed the presence of structures rich in N-acetylgalactosaminyl termini (Table 4), and possibly their sialylated forms. These findings are consistent with the results of McMahon *et al*, who found increased expression of WFA and HPA ligands and down regulation of VVA ligands [9]. While, Arenas *et al* found weak HPA staining [6], which might be a result of using different disclosing methods from McMahon *et al* and this current study.

WFA binds to GalNAcα1,6Galβ1- with an affinity about fifty times greater than that it shows to GalNAcα1,3Galβ1- on both type I and II chains. Mass action versus affinity to WFA ligands, in this study, seemed to be very commonly expressed in the transformed prostatic epithelial cells in prostate tumours. In comparison, VVA, which has a much higher affinity for GalNAcα1,3Galβ1- than GalNAcα1,6Galβ1-, showed a dramatic down-regulation in the expressions of VVA ligands. This argues that, WFA is largely binding to α1, 6 linked ligands. In addition, the increase in WFA and VVA staining after pre-treatment with dilute acid shows that the synthetic pathway for the carbohydrate sequence GalNAcα1,6Galβ1- was highly active in prostatic carcinoma. Dilute acid pre-treatment revealed the presence either of sialylated forms of the WFA and VVA ligands, or that at least some of the WFA and VVA ligands are hindered by other sialyl glycans, or both.

HPA specifically binds terminal 2deoxy, 2acetamidogalactosyl residues in sugar chains (for example, Tn antigen and histo-blood group A determinant and DBA ligands). Although HPA belongs to a protein family unrelated to VVA and WFA, it can, to a lesser extent, recognise the GalNAc residues of the WFA and VVA ligands. This may well explain the differences in staining patterns between HPA and both WFA and VVA. However, because of the diminished staining with DBA and VVA, it is more likely that the strong staining of HPA is a result of increased synthesis of Tn antigen and, perhaps, WFA ligands. This was also confirmed by the observations after the dilute acid pre-treatment, which showed also the presence of sTn antigen and the sialylated type of GalNAcα1-6Galβ1-3/4GlcNAcβ1-. These are consistent with the observations of Zhang *et al*, who used antibodies against Tn and sTn antigens and found their expression in almost all cases of prostatic carcinoma [16].

BSA-1_{B4} lectin showed the presence of Galα1, GalαA1-3(Fucα1-2) Galβ1-4GlcNAc- and Galα1-3Gal-, especially after pre-treatment with dilute acid [11]. However, MPA and ECA staining confirmed, to some extent, the presence of Galα1- and Galα1-3Galβ1-4GlcNAcβ1- respectively.

In MPA, ECA, CTA and AHA group of lectins mainly confirms the occurrence of several different

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Table 4. The carbohydrate structures recognised by the combination of markers of the present study.

Group	Markers	Accepter
Group 1	AAA	Fucα1-2Gal β1-3GlcNAcβ1-
	UEA-1	Fucα1-2Gal β1-4GlcNAcβ1-
	DBA	GalNAcα1-3(Fucα1-2)Gal β1-3/4GlcNAcβ1-
	MAb Le^a	Gal β1-3(Fucα1-4)GlcNAcβ1-3Gal
	MAb sLe^a	NeuAcα2-3Gal β1-3(Fucα1-4)GlcNAcβ1-3Gal
	MAb sLe^x	NeuAcα2-3Gal β1-4(Fucα1-3)GlcNAcβ1-3Gal
Group 2	WFA, VVA, HPA	GalNAcα1-6Galβ1-
	VVA, HPA, WFA	GalNAcα1-3Galβ1-
	HPA	GalNAcα1-3 (Fucα1-2)Galβ1-3/4GlcNAc, and GalNAc α-Ser/Thr
Group 3	$BSA\text{-}1_{B4}$	Galα1- & Galα1,3Gal-
Group 4	MPA	Galα1- and GalNAcα1-
	MPA, AHA	Galβ1-3GalNAcα1-
	ECA, $BSA\text{-}1_{B4}$	Galα1-3Galβ1-4GlcNAcβ1-
	AHA, ECA, CTA	Galβ1-4GlcNAcβ1-
	CTA	Galβ1-4GlcNAcβ1- especially in multiple branches
Group 5	ECA, CTA, MAA	NeuAcα2-3Galβ1-4GlcNAc-
	CTA, MAA, $siLe^x$	NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc-
	HPA, WFA, VVA, MPA, SNA	NeuAcα2-6(GalNAcα1-3)Galβ1-3/4GlcNAc
	HPA, WFA, VVA, MPA, MAA	NeuAcα2-3(GalNAcα1-3)Galβ1-3/4GlcNAc-
	WFA, HPA, MPA, SNA	NeuAcα2-6(GalNAcα1-6)Galβ1-3/4GlcNAc-
	WFA, HPA, MPA, MAA	NeuAcα2-3(GalNAcα1-6)Galβ1-3/4GlcNAc-
	MAA, sLe^a	NeuAcα2-3Galβ1-3(Fucα1-4)GlcNAc-
	AHA, MPA, MAA	NeuAcα2-3Galβ1-3GalNAc-
	AHA, MPA, SNA	NeuAcα2-6Galβ1-3GalNAc-
	HPA, MPA, SNA	NeuAcα2-6GalNAc-
	ECA, CTA, SNA	NeuAcα2-6Galβ1-4GlcNAc-
Group 6	GNA	Manα1,3Man
	NPA	Manα1,6Man

Fuc: fucose; Gal: galactose; GlcNAc: 2 deoxy, 2 acetamidoglucosyl residue; GalNAc: 2 deoxy, 2 acetamidogalactosyl residue; NeuAc: sialic acid; Man: mannose; MAb Le^a: monoclonal antibody against $Lewis^a$ antigen; MAb sLe^a: monoclonal antibody against sialyl $Lewis^a$ antigen; MAb sLe^x: monoclonal antibody against sialyl $Lewis^x$ antigen.

types of galactosylated glycans in carbohydrate sequences expressed on transformed prostatic epithelium. These observations are consistent with the findings of Foster *et al* [21], and McMahon *et al* [9] for ECA staining, Soderstrom [8], McNeal & Alroy *et al* [11], Foster *et al* [21], McMahon *et al* [9], Drachenberg & Papadimitriou [12], Janssen *et al* [22] and Arenas *et al* [6] for AHA staining. The data-analysis of this present study suggests that there is increased synthesis of the precursor of type II chain Gal 1-4GlcNAc in long and multiply branched carbohydrate chains, followed by increased galactosylation and 2-deoxy-2-acetamidogalactosylation. There is also increased synthesis of the structure Galβ1-3GalNAcα1- (core 3).

The results of using MAA and SNA in the present study clearly demonstrate the presence of hypersialylation, which is a common observation in transformed cells in many kinds of tumours including prostatic carcinoma [6,23-26]. However, McMahon *et al* [9] found increased expression of SNA ligands, but not MAA ligands. This finding, in regard to MAA, does not contradict the observations of this present study, as it was found that there was a group of cases of prostatic carcinoma (4 out of 27) which did not bind to MAA but

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bound to either sLe^x or sLe^a or to both of them. This means that expression of NeuAcα2-3- was present, but was not recognised by MAA, most probably because of steric constraints caused by the fucosyl residues of sLe^x and sLe^a structures. Nevertheless, the findings of this study allow for the confident prediction of the possible presence of certain sialylated structures listed in Table 4. The results, in the present study, indicated that the main biosynthetic pathways of sialylation are through α2-3 and α2-6 linkages, synthesised by α3-sialyltransferase (ST3Gal) and α6-sialyltransferase (ST6Gal) families respectively.

Two lectins, GNA and NPA, showed the presence of α-D-mannose residues in prostatic carcinoma, especially in Man 1-3Man and Manα1-6Man linkages respectively. This is consistent with the findings of Arenas *et al* [6] who also found strong staining by GNA and NPA in transformed prostatic cells. The best explanation is that α-D-Man residues were added in both linkages to immature N-glycans sequences in early stages of their biosynthesis in endoplasmic reticulum or the Golgi apparatus. These N-glycans then were subjected to further modifications, by the actions of, first, mannosidases and, then, glycosyltransferases, which added different glycosyl residues such as Gal, GalNAc, GlcNAc, Fuc and sialic acids. This explanation is supported by the location of staining in the apical cytoplasm and in the supra-nuclear region (Fig. 17), and also the results of staining by other lectins used in this present study, especially after β-elimination which showed that the changes of glycosylation observed were present on both O- and N-glycans. An alternative explanation is that the accumulation of lectin-binding material in the cytoplasm may be indicative of acceleration in synthesis, or a breakdown in transport mechanisms, so that the carbohydrate structures are being produced faster than they could be transferred to the cell surface.

Lectin binding to prostatic carcinoma following β-elimination, showed that most of the carbohydrate structures demonstrated by the lectins used in this study are carried on O-glycans, as there was a dramatic decrease in staining intensity after β-elimination, except for WFA, BSA-1_{B4}, GNA, NPA, MAA and SNA, which showed increased or unchanged staining in some cases (Table 3). A likely explanation for these results is that the steric hindrance caused by O-glycans has been removed, thus much more N-glycans have been recognised by lectins. Alternatively, the ligands of these lectins, especially for GNA and NPA, are carried only on N-glycans. However, the same changes of glycosylation observed on O-glycans also occurred on N-glycans since some staining was retained following β-elimination. All these changes occurred on the terminal parts of the O- and N-glycans because most of the lectins, if not all, bind to terminal residues.

A great number of the carbohydrate structures demonstrated in this study were sialylated either through α2-3 or β2-6 linkages, since staining by most lectins was considerably increased following dilute acid pre-treatment, except for SNA and MPA and in some cases WFA, MAA, GNA and NPA, which showed slightly increased or unchanged staining. It is, thus, possible that staining level of some lectins may remain the same as a result of 'balanced losses, i.e., losing long chain glycans but revealing hidden short chain glycans. So, there is no change in the overall level of staining, but there is a change in the distribution pattern of binding among the cell populations in the tissue sections. Another explanation is that the presence sialyl residues do not restrict the recognition of glycans by lectins.

Conclusion

The findings of this study showed that the glycan phenotype of transformed prostatic cells is different from their normal counterparts. The malignant prostatic cells had a distinct glycophenotype, which was demonstrated by an increase of fucosylation, galactosylation, 2 deoxy, 2 acetamide galactosylation or masking of carbohydrate residues. Lectin staining, in this present study, enabled both the accurate identification of cells displaying the marker in question and, in addition, allowed one to determine whether the particular marker is intracellular or membranous. Much of the variation in binding appeared to be related to cellular differentiation and maturation. Some lectins showed, to some extent, focal and patchy staining, in some if not all cases. This finding suggests that transformed prostatic cells, which appeared to be of equivalent differentiation and maturation, and appeared to be morphologically identical actually displayed different cell surface glycoconjugates. This might reflect cells with differing functions and capabilities. This is especially important when studying heterogeneous tissues such as epithelial lesions, where expression may vary between the various tissue components.

Acknowledgements: This work was funded by Overseas Research Students Awards Scheme and the University of Manchester

References

[1] Ogawa J, Sano A, Inoue H, Koide S. Expression of Lewis-related antigen and prognosis in stage I non-small cell lung cancer. *Ann Thorac Surg.* 1995;59:412-415.
[2] Hakomori S. Aberrant glycosylation in tumours and tumour-associated carbohydrate antigens. *Adv Cancer Res.* 1989;52: 257-331.
[3] Ito N, Hirota T. Histochemical and cytochemical localization of blood group antigens. *Prog Histochem Cytochem*. 1992; 25:1-85.

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[4] Jones CJ, Stoddart RW. A post-embedding avidin-biotin peroxidase system to demonstrate the light and electron microscopic localization of lectin binding sites in rat kidney tubules. *Histochem J*. 1986;18:371-379.
[5] Downs F, Herp A, Moschera J, Pigman W. Beta-elimination and reduction reactions and some applications of dimethylsulfoxide on submaxillary glycoproteins. *Biochim Biophys Acta*. 1973;328:182-192.
[6] Arenas MI, Romo E, de Gaspar I, de Bethencourt FR, Sánchez-Chapado M, Fraile B, Paniagua R. A lectin histochemistry comparative study in human normal prostate, benign prostatic hyperplasia, and prostatic carcinoma. *Glycoconj J*. 1999;16:375-382.
[7] Abel PD, Keane P, Leathem A, Tebbutt S, Williams G. Change in glycoconjugate for the binding site of the lectin Ulex europeus 1 following malignant transformation of prostatic epithelium. *Br J Urol*. 1989;63:183-185.
[8] Söderstrom KO. Lectin binding to prostatic adenocarcinoma. *Cancer*. 1987;60:1823-1831.
[9] McMahon RF, McWilliam LJ, Clarke NW, George NJ. Altered saccharide sequences in two groups of patients with metastatic prostatic carcinoma. *Br J Urol.* 1994;74:80-85.
[10] Loy TS, Kyle J, Bickel JT. Binding of soybean agglutinin lectin to prostatic hyperplasia and adenocarcinoma. *Cancer*. 1989;63:1583-1586.
[11] McNeal JE, Alroy J, Villers A, Redwine EA, Freiha FS, Stamey TA. Mucinous differentiation in prostatic adenocarcinoma. *Hum Pathol*. 1991;22:979-988.
[12] Drachenberg CB, Papadimitriou JC. Aberrant pattern of lectin binding in low and high grade prostatic intraepithelial neoplasia. *Cancer*. 1995;75:2539-2544.
[13] Young WW Jr, Mills SE, Lippert MC, Ahmed P, Lau SK. Deletion of antigens of the Lewis a/b blood group family in human prostatic carcinoma. *Am J Pathol.* 1988;131:578-586.
[14] Perlman EJ, Epstein JI. Blood group antigen expression in dysplasia and adenocarcinoma of the prostate. *Am J Surg Pathol*. 1990;14:810-818.
[15] Idikio HA, Manickavel V. Correlation of blood group antigen expression and oncogene-related proteins in malignant prostatic tissues. *Pathol Res Pract*. 1991;187:189-197.
[16] Zhang S, Zhang HS, Cordon-Cardo C, Reuter VE, Singhal AK, Lloyd KO, Livingston PO. Selection of tumour antigens as targets for immune attack using Immunohistochemistry: II. Blood group-related antigens. *Int J Cancer*. 1997;73:50-56.
[17] Jorgensen T, Berner A, Kaalhus O, Tveter KJ, Danielsen HE, Bryne M. Up-regulation of the oligosaccharide sialyl Lewis^x: a new prognostic parameter in metastatic prostate cancer. *Cancer Res*. 1995;55:1817-1819.
[18] Martensson S, Bigler SA, Brown M, Lange PH, Brawer MK, Hakomori S. Sialyl-Lewis(x) and related carbohydrate antigens in the prostate. *Hum Pathol*. 1995;26:735-739.
[19] Jorgensen T, Kanagasingam Y, Kaalhus O, Tveter KJ, Bryne M, Skj?rten F, Berner A, Danielsen HE. Prognostic factors in patients with metastatic (stage D2) prostate cancer: experience from the Scandinavian Prostatic Cancer Group Study-2. *J Urol*. 1997;158:164-170.
[20] Idikio HA. Sialyl-Lewis-X, Gleason grade and stage in nonmetastatic human prostate cancer. *Glycoconj J*. 1997;14:875-877.
[21] Foster CS, McLoughlin J, Bashir I, Abel PD. Markers of the metastatic phenotype in prostate cancer. *Hum Pathol*. 1992;23:381-394.
[22] Janssen T, Petein M, Van Velthoven R, Van Leer P, Fourmarier M, Vanegas JP, Danguy A, Schulman C, Pasteels JL, Kiss R. Differential histochemical peanut agglutinin stain in benign and malignant human prostate tumours: relationship with prostatic specific antigen immunostain and nuclear DNA content. *Hum Pathol.* 1996;27:1341-1347.
[23] Fukuda M. Possible roles of tumour-associated carbohydrate antigens. *Cancer Res*. 1996;56:2237-2244.
[24] Hakomori S. Tumour-associated carbohydrate markers. In: *Serological cancer markers*, Sell S (Ed) Human Press, New Jersey, 1992, pp 207-232.
[25] Aubert M, Panicot L, Crotte C, Gibier P, Lombardo D, Sadoulet MO, Mas E. Restoration of alpha (1, 2) fucosyltransferase activity decreases adhesive and metastatic properties of human pancreatic cancer cells. *Cancer Res*. 2000;60:1449-1456.

Submitted: 28 May, 2010
Accepted after reviews: 8 August, 2010

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